SILVA BALCANICA

Silva Balcanica 22(3): 49-55 (2021)
doi: 10.3897/silvabalcanica.22.e77816

RESEARCH ARTICLE

Acute toxicity of *Galium odoratum* to the freshwater cladoceran *Moina macrocopa*

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Academic editor: Alexander Delkov | 9 November 2021 | 10 December 2021 | Published 28 December 2021

Citation: Bozhilova, M. 2021. Acute toxicity of *Galium odoratum* to the freshwater cladoceran *Moina macrocopa*. Silva Balcanica 22(3): 49-55. https://doi.org/10.3897/silvabalcanica.22.e77816

Abstract

Galium odoratum (L.) is a medicinal plant with a number of health benefits, used in traditional and modern medicine. The toxicity of the coumarin in *Galium odoratum* is classified as high, however, no experimental data or data on toxic effects in humans following intake of *Galium odoratum* are available. The potential toxic effect can be estimated based on the content of coumarin and knowledge of its toxicity.
The aim of the current study is to evaluate the acute toxicity effects of a range of concentrations of *Galium odoratum* water extract on *Moina macrocopa* and calculate the LC_{50} within 24 hours. In order to compare the toxicity with those of other, well-known and widely used medicinal plants, extracts of *Matricaria chamomilla* and *Tribulus terrestris* are also tested.
The results show that LC_{50} value of *Galium odoratum* is comparable with those of *Matricaria chamomilla* and *Tribulus terrestris,* and *Galium odoratum* has intermediate toxicity between the two other studied species.

Keywords

Galium odoratum, *Moina macrocopa*, toxicity, LC_{50}

Introduction

Galium odoratum (L.) is a perennial herbaceous plant, naturally occurring in Europe, Asia and Northern Africa. It is used for centuries in traditional medicine for the treatment of central nervous system problems and disorders (Wszelaki et al., 2010; Kahkeshani et al., 2013; Friscic et al., 2018a), stomach, liver and digestive problems (Vlase et al., 2014; Mocan et al., 2016; Friscic et al., 2018a), gout treatment (Mocan et al.,

2016), etc. It is valued for its antihypoxic (Iurchenko et al., 2015) and antimicrobial effect (Wagnera et al., 2017), ability to cleanse the blood (Friscic et al., 2018a), improve metabolism (Ilyina et al., 2016) and memory (Wszelaki et al., 2010), reduce swelling, cure wounds and cuts (Kahkeshani et al., 2013; Vlase et al., 2014; Wagnera et al., 2017).

Galium odoratum is used in modern medicine. Its proven pharmacological properties include antioxidant (Friscic et al., 2018b), antimicrobial, antifungal and antiviral effect (Cowan, 1999; Wojnicz et al., 2012; Vlase et al., 2014), burn wound healing activity (Kahkeshani et al., 2013), anti-hypoxic and sedative effect (Wojnicz et al., 2012; Iurchenko et al., 2015). The species is rich in biologically active compounds, including coumarin, asperulosiode, monotropein, scandoside, rutin, quercitrin, etc. (Wolf, 1993; Kahkeshani et al., 2013; Vlase et al., 2014).

The toxicity of the coumarin in *Galium odoratum* is classified as high (Duke, 1985; Cowan, 1999). The species is included in lists of toxic plants, e.g. Ordinance No. 1/12.01.2009, List of substances of FGFSA (2014), etc. However, no experimental data or data on toxic effects in humans following intake of *Galium* odoratum are available. The potential toxic effect can be estimated based on the content of coumarin and knowledge of its toxicity (BfR Health Assessment No 044, 2006; Egebjerg et al., 2018).

Cladoceran species are used in toxicity tests due to their short life cycle, easy cultivation in the laboratory, sensitivity to toxicants, small size requiring small volumes of extracts for testing, etc. Acute toxicity tests with *Moina macrocopa* are widely applied in ecotoxicology (Chu et al., 1997; Ji et al., 2008; Nam et al., 2009; Yi et al., 2010).

The aim of the current study is to evaluate the acute toxicity effects of a range of concentrations of *Galium odoratum* water extract on *Moina macrocopa* and calculate the LC_{50} (concentration lethal to 50% of the test animals) within 24 hours. In order to compare the toxicity with those of other, well-known and widely used medicinal plants, extracts of *Matricaria chamomilla* and *Tribulus terrestris* were also tested.

Materials and Methods

Galium odoratum aboveground biomass was collected in the summer of 2020 (during blossoming) from 8 localities in Mala and Ponor Mountains, Bulgaria. The row material was thoroughly mixed, air-dried and chopped. Dry aboveground biomass of *Matricaria chamomilla* and *Tribulus terrestris* was purchased from a recognized producer of Bulgarian medicinal plants.

50 g of dry plant material from each species were submerged in 1000 ml boiling water and left to macerate for 24 h. After maceration, the plant material was filtered, and the extracts were used for the preparation of the test solutions.

Series of concentrations were selected from the lists available in Report EPS 1/RM/11 (1990). The tested concentrations are listed in Table 1.

For the toxicity test were used *Moina macrocopa* neonates (less than 24 hours old) from a laboratory culture maintained in a climate-controlled room at 25 ± 2°C, on a 16:8 h light/dark photoperiod for 2 weeks before testing. Animals were handled as

Table 1. Series of concentration used for the toxicity test

Extract (ml) in 100 ml test solution	Concentration (mg/ml) in the test solution
1.00	0.50
1.80	0.90
3.20	1.60
5.60	2.80
10.00	5.00
18.00	9.00
32.00	16.00
56.00	28.00
100.00	50.00

little as possible, carefully, and quickly to minimize stress. Dechlorinated municipal water was used for cultivation, dilution and control. During cultivation, animals were fed dry yeast once every 24 h.

Neonates for the tests were obtained as gravid females from the culture were transferred to another vessel 24 hours before the test and fed, in order to increase the number of reproducing animals. Neonates were held in the test solutions for 24 hours. They were not fed during the test. At the end of the test period, the living individuals in the control and test solutions were counted. The LC_{50} values and their 95% confidence intervals (CI) were calculated using Probit Analysis, following the methodology of Finney (1971) in Excel 2010 (Mekapogu, 2021). The data for mortality proportions were transformed to probits, and the respective concentrations – to log_{10}. The LC_{50} values were estimated using regression analysis (Busvine, 1971). The original mortality (observed) and derived mortality (expected) were used to calculate the Chi-Square test. The goodness of fit was assessed via the R^2 value.

Results and Discussion

The survival rate of *Moina macrocopa* in the control was 100%. The survival rates in the tested concentrations of *Galium odoratum, Matricaria chamomilla* and *Tribulus terrestris* are given in Table 2.

The mortality increased with the increase in the concentration of plant extracts according to the mortality concentration curves shown in Figure 1.

LC_{50} values of the water extracts of the studied medicinal plants, calculated using probit analysis, are given in Table 3.

For all three plant species, the concentration of the extract had an inversely proportional relationship with the survival rate of *Moina macrocopa*. In the samples with concentrations 0.5 and 0.9 mg/ml, as well as in the control, the survival rate was 100%. In the *Matricaria chamomilla* extract, all water fleas survived also at 1.6 mg/ml and 2.8 mg/ml. In extracts with concentrations of 28 and 50 mg/ml there were no

Table 2. Survival rates for 24 h exposure of *Moina macrocopa* to aqueous extract of *Galium odoratum, Matricaria chamomilla* and *Tribulus terrestris*

Test solution (mg/ml)	Survival rate (%)		
	Galium odoratum	***Matricaria chamomilla***	***Tribulus terrestris***
Control	100	100	100
0.5	100	100	100
0.9	100	100	100
1.6	80	100	80
2.8	80	100	60
5	70	80	60
9	60	60	0
16	20	20	0
28	0	0	0
50	0	0	0

Table 3. LC_{50} of *Galium odoratum, Matricaria chamomilla, Tribulus terrestris*

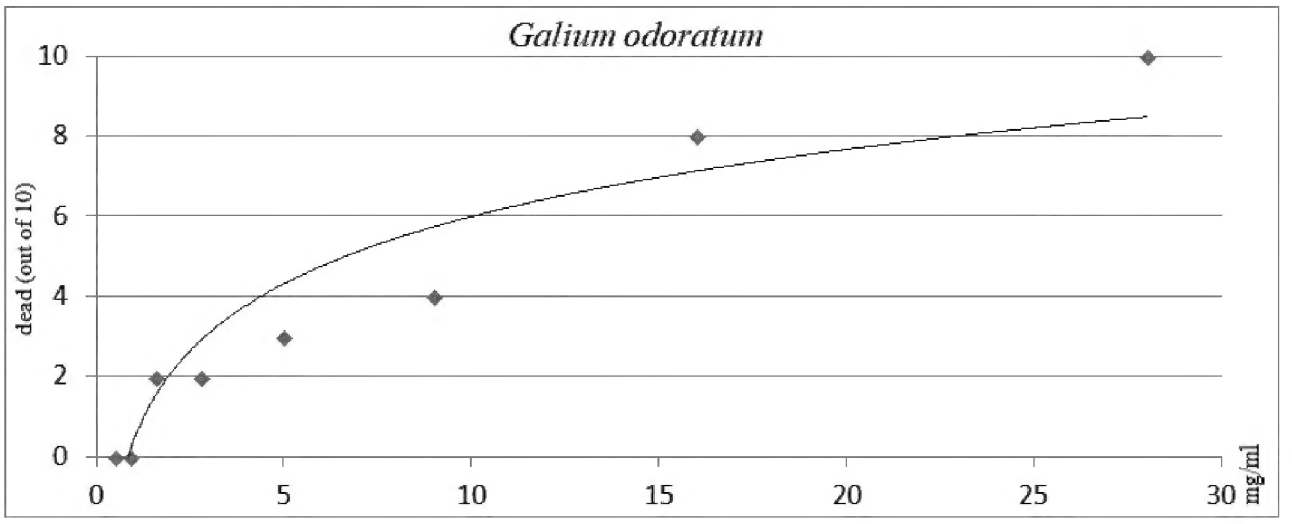
Species	LC_{50} (mg/ml)	95% CI		R^2
		Lower	Upper	
Galium odoratum	8.18	5.01	13.40	0.96
Matricaria chamomilla	11.59	7.83	17.16	0.97
Tribulus terrestris	6.74	3.71	12.20	0.93

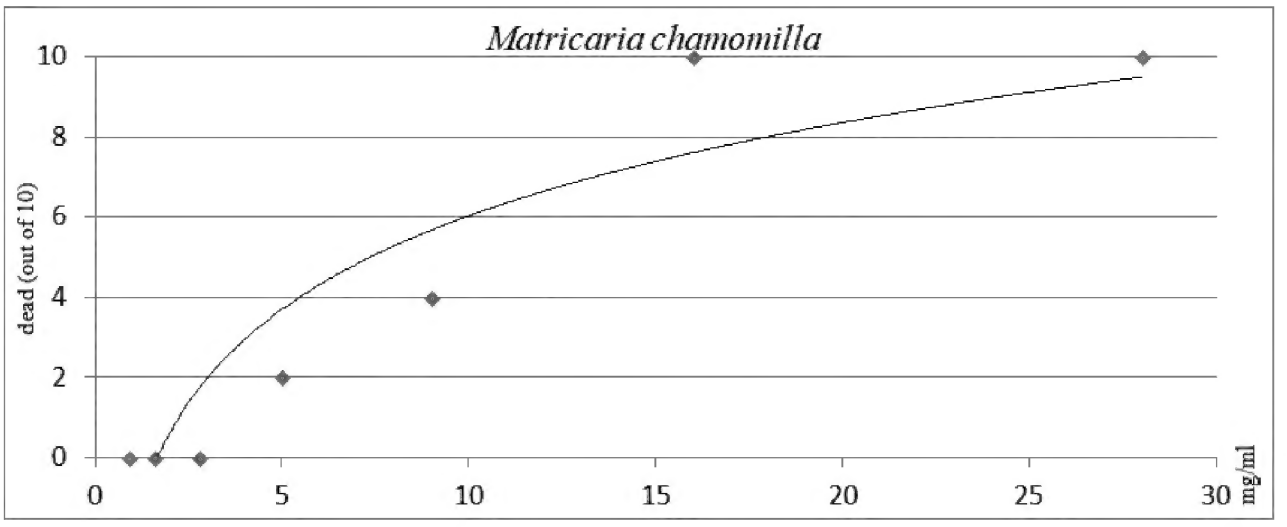
living water fleas. In the extracts of *Tribulus terrestris* there were no living individuals also at concentrations 9 mg/ml and 16 mg/ml.

Based on the toxicity tests with *Moina macrocopa* can be concluded that *Galium odoratum* has intermediate toxicity between *Matricaria chamomilla* and *Tribulus terrestris.* The LC_{50} of *Galium odoratum* is 8.18 mg/ml with a 95% CI 5.01-13.40 mg/ml. The highest toxicity of the three studied plant species was observed in *Tribulus terrestris* extracts. The LC_{50} is 6.74, with a 95% confidence limit 3.71-12.20 mg/ml. *Matricaria chamomilla* showed the lowest toxicity with LC_{50} 11.59 mg/ml with a 95% confidence limit of 7.83-17.16 mg/ml. Based on the results of the Chi-tests (non-significant), the observed and derived mortality showed a good fit.

Conclusion

The LC_{50} value of *Galium odoratum* is 8.18 mg/ml with a 95% CI 5.01-13.40 mg/ml and is comparable with those of *Matricaria chamomilla* and *Tribulus terrestris.* It is 30% lower than the LC_{50} of *Matricaria chamomilla* and 18% higher than this of *Tribulus terrestris,* i.e. the toxicity of *Galium odoratum* is higher than the toxicity of *Matricaria chamomilla* and lower than that of *Tribulus terrestris.*





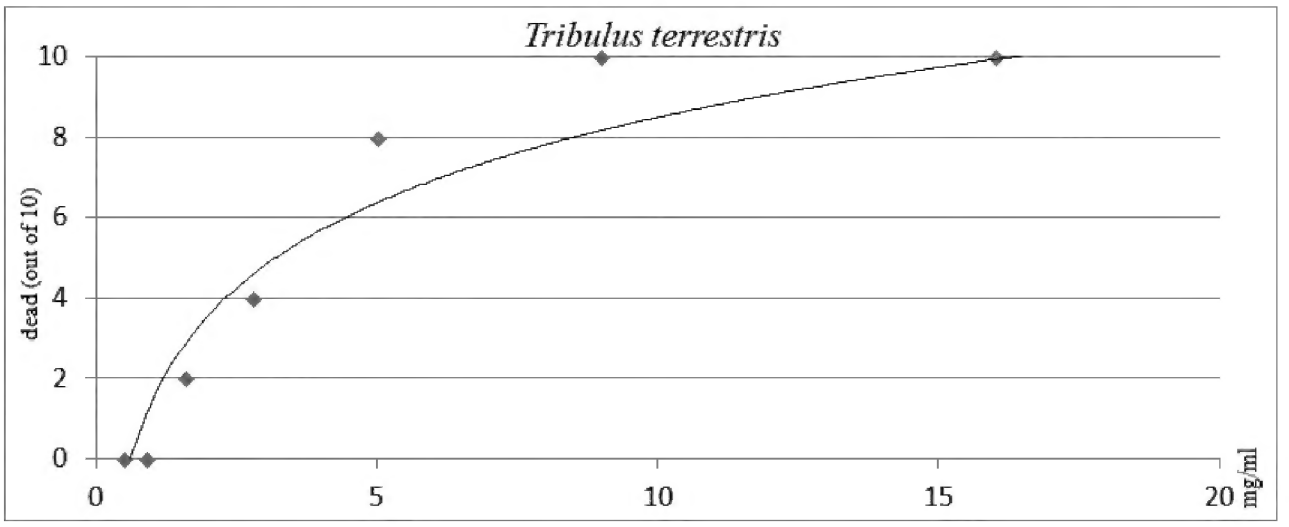


Figure 1. Mortality concentration curves (logarithmic trend line) for 24 h exposure of *Moina macrocopa* to aqueous extract of *Galium odoratum, Matricaria chamomilla* and *Tribulus terrestris*

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